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cVEMP morphology changes with recording electrode position but single motor unit activity remains constant

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Abstract

Cervical vestibular evoked myogenic potentials (cVEMPs) recorded over the lower quarter of the sternocleidomastoid (SCM) muscle in normal subjects may have opposite polarity to those recorded over the midpoint. It has thus been suggested that vestibular projections to the lower part of SCM might be excitatory rather than inhibitory. We therefore tested the hypothesis that the SCM muscle receives both inhibitory and excitatory vestibular inputs. We recorded cVEMPs in 10 normal subjects with surface electrodes placed at multiple sites along the anterior (sternal) component of the SCM muscle. We compared several reference sites: sternum, ipsilateral and contralateral earlobes and contralateral wrist. In five subjects, single motor unit responses were recorded at the upper, middle and lower parts of the SCM muscle using concentric needle electrodes. The surface cVEMP had the typical positive-negative polarity at midpoint of the SCM muscle. In all subjects, as the recording electrode was moved toward each insertion point, p13 amplitude became smaller and p13 latency increased, then the polarity inverted to a negative-positive waveform (n1-p1). Changing the reference site did not affect reflex polarity. There was a significant short-latency change in activity in 61/63 single motor units and in each case this was a decrease or gap in firing, indicating an inhibitory reflex. Single motor unit recordings showed that the reflex was inhibitory along the entire SCM muscle. The cVEMP surface waveform inversion near the mastoid and sternal insertion points likely reflects volume conduction of the potential occurring with increasing distance from the motor point.

Introduction

Vestibular evoked myogenic potentials (VEMPs) are muscle reflexes elicited by activation of the vestibular system with short bursts of sound, vibration or galvanic stimulation. They are most commonly recorded in the sternocleidomastoid (SCM) neck muscles in response to stimulation of the ipsilateral ear with loud air-conducted (AC) clicks or tone bursts (cervical VEMPs, or cVEMPs) (4). The cVEMP is recorded using an active surface electrode placed over the middle of the SCM muscle belly, near the motor point, and a reference over the medial clavicle. Under these stimulation and recording conditions the cVEMP consists of a short-latency, biphasic positive-negative potential with peak latencies of approximately 13 and 23 ms (i.e. p13-n23).

The p13-n23 surface potential has been shown to result from a brief inhibition of the SCM muscle. Colebatch and Rothwell (5) recorded the responses of single motor units in SCM muscles in normal volunteers and found that the surface cVEMP to ipsilateral AC click stimulation was produced by a brief decrease or gap in motor unit firing. This inhibitory effect is thought to originate predominantly from irregularly-firing otolith afferents (6). Afferents from all vestibular organs respond to sound stimulation, but those with the lowest thresholds are found in the saccule, followed by the utricle (23, 24). All vestibular organs appear to have inhibitory projections to the ipsilateral SCM muscle, while the utricle and semicircular canals have additional excitatory projections to the contralateral SCM (18). As contralateral responses are rarely seen in normal subjects, the cVEMP is considered to be an oligosynaptic inhibitory reflex originating predominantly in the ipsilateral saccule.

A recent study in healthy human subjects by Wei et al. (20) made the interesting observation that cVEMPs recorded from the lower quarter of the SCM muscle have opposite polarity to those recorded over the midpoint and upper quarter of the muscle. The inverted reflexes were smaller than those recorded over the muscle midpoint, but showed the same stimulus frequency preference. Previous studies have similarly recorded cVEMPs from near

92 the insertion points of the SCM muscle, but have not shown clear polarity inversions.
93 Sheykholeslami et al. (16) recorded from sites close to the mandibular angle and the sternal
94 and clavicular SCM muscle origins and reported regular p13-n23 responses in all cases except
95 5 subjects at the sternal muscle head. However, they did not specifically report a polarity
96 inversion. Colebatch (3) recorded at multiple locations along and beside the SCM muscle
97 using two different reference sites, and found a polarity inversion for the electrodes close to
98 the sternal insertion point, but only when the reference was over the C7 spinal process. It was
99 thought that this might have been caused by interference from the 'inion response' (2), which
100 originates in the posterior neck muscles and might contaminate the C7 reference (3).
101 Based on their data, Wei et al. (20) suggested that the vestibular projections to SCM
102 may not be uniform across the length of the muscle and that the projection to the lower part of
103 SCM might be excitatory rather than inhibitory. Some animal species show
104 compartmentalization of long muscles with multiple innervation zones (e.g. 22), however the
105 purpose of compartmentalization is thought to be to make propagation of a single signal more
106 efficient. Wei et al. (20) alternatively hypothesised that volume conduction of the motor unit
107 action potentials could lead to a polarity inversion near the muscle-tendon junction.
108 We aimed to further investigate the polarity inversion of the cVEMP and test the
109 hypothesis that the SCM muscle receives both excitatory and inhibitory vestibular inputs. We
110 recorded cVEMPs with surface electrodes placed at multiple sites along the anterior
111 component of the SCM muscle and compared several reference sites: the sternum, ipsilateral
112 and contralateral earlobes and contralateral wrist. In a subset of five subjects, single motor
113 unit responses were recorded at the upper, middle and lower parts of the SCM muscle using
114 concentric needle electrodes. Single motor unit recordings were performed since they provide
115 unequivocal information about the polarity of the reflex at the recording location, while
116 surface potentials are affected by electrode placement relative to underlying muscle structures,

such as the motor point and tendons. We also measured the SCM sternal tendon with ultrasound to relate the surface cVEMP changes to the muscle structure.

Materials and Methods

Subjects

Twelve normal volunteers with no history of conductive hearing loss or vestibular or neurological disease participated (6 females, 6 males; age range 24-66 years). Participants were staff and research associates of Royal Prince Alfred Hospital, Sydney, and all gave written informed consent according to the Declaration of Helsinki. The study was approved by the local ethics committee (X13-0270 & HREC/13/RPAH/354).

Vestibular stimulation

The stimulus was a 500 Hz burst of 2 ms duration (0 ms rise/fall) delivered with calibrated headphones (TDH 39, Telephonics Corp., Farmingdale, USA) and custom amplifier at 130-136 dB peak SPL (102 to 108 dB L_{Aeq}). The stimuli were generated with Signal software using a laboratory interface (micro1401, Cambridge Electronic Design (CED)) and delivered at a rate of 5 Hz. For the surface-only recordings 200 stimuli were delivered, while for the single motor unit recordings there were usually 1200 stimuli, ranging up to 1500.

Surface cVEMP recordings

Surface recordings were made in ten of the subjects to track the changes in cVEMP with different electrode positions along the muscle and to test the effect of different reference electrode positions (6 females, 4 males; age range 24-66 years). We first measured the length of the sternomastoid muscle from its origin at the mastoid (measured from a point directly posterior to the external auditory meatus) to the insertion at the sternum and marked the 25, 50 and 75% points along the muscle to use as guide points. During the SCM measurement

143 subjects were seated and turned their heads slightly away from the measured side, while
144 during testing they reclined supine and lifted and turned their heads by the same degree.
145 Using a sternal reference, to replicate the montage used by Wei et al. (20), we recorded
146 cVEMPs with an active electrode placed at the midpoint. We then moved the active electrode
147 to either the 25% or the 75% mark (in counterbalanced order) and systematically tested at,
148 above and below these guide points in 1 cm increments to determine whether the surface
149 response changed polarity and at which point this occurred. A different number of recordings
150 was performed in each subject to enable efficient identification of the reflex inversion point
151 (median 10, range 6-16). At the upper and lower parts of the muscle we marked the positions
152 where the cVEMP polarity was clearly inverted. We then systematically compared the
153 cVEMPs recorded at these positions and the midpoint using three additional reference
154 electrode sites: the contralateral wrist, contralateral earlobe and ipsilateral earlobe.
155 EMG activity was recorded with the same micro1401 data acquisition interface and
156 Signal software as described above and 1902 amplifiers (mk4, CED). For the surface-only
157 recordings, data were amplified and sampled at 10 kHz from 20 ms before to 80 ms after
158 stimulus onset. Filter settings were (5 Hz to 2 kHz). For the combined surface-single unit
159 recordings data were sampled at 50 kHz in continuous frames of 200 ms (from 100 ms before
160 to 100 ms following stimulus onset) and filter settings were 5 Hz to 10 kHz. Negative
161 potentials at the active electrodes were displayed as upward deflections.
162
163 *Single motor unit recordings*
164 Single motor unit recordings were performed in a separate session in a subset of 5 of
165 the above subjects (2 females, 3 males, 37-66 yrs of age). We recorded motor units at 3 sites
166 along the sternomastoid muscle: in the middle of the muscle belly and as close as possible to
167 the tendons at the mastoid and sternal head. We measured the muscle as above and again
168 marked the 25, 50 and 75% points. We made multiple recordings near each of these sites in

each subject. For the 25 and 75% points, the needle was inserted as close to the tendon as possible, determined by palpating the muscle, and so did not always strictly follow the markers. Recordings were made in either the left (2 subjects) or right (3 subjects) SCM according to subject preference or side of better response and were always ipsilateral to the stimulated ear. In one subject, in a separate session, additional recordings were made very close to the sternal head of SCM to test the polarity of the response at a more extreme distal part of the muscle. The needle was inserted laterally, behind the more superficial tendinous part of the muscle, 16 cm below the mastoid in the right SCM (3 cm above the clavicle, 85% of the total muscle length). This site was below the site of cVEMP inversion for this subject, which was at 14.5 cm below the mastoid.

We used ultra thin (0.3 mm diameter, 30G) disposable concentric needles (Neuroline, Ambu A/S) to record single motor units. After preparing the skin with 70% isopropyl alcohol, a needle electrode was inserted and held in position with tape during each recording. Subjects sat upright, turned their head slightly away from the recorded side and produced a weak contraction of the SCM by pushing gently against their chin with their hand. Audio and visual feedback was provided to help subjects maintain constant activation of one or more motor units. Surface potentials were recorded simultaneously using an active electrode placed near the needle electrode, a reference electrode on the sternum and an earth on the lateral clavicle. EMG activity was recorded as described above.

Comparison with electrically-evoked compound muscle action potential

In five of the subjects (2 females, 3 males, 24-60 yrs of age), we recorded electrically-evoked compound muscle action potentials (CMAPs) in SCM to investigate whether this reflex also inverted with changing electrode position. We stimulated with approximately 100 pulses of 4-7 mA and 0.1 ms duration using a ball electrode applied close to the motor point near the midpoint of the right SCM muscle. The stimulus was applied either to the motor

point directly or slightly posterior to the muscle, over the portion of accessory nerve innervating the anterior component of SCM selectively. A custom artefact eliminator was used from 0.5 ms before to 1.5-2 ms after stimulus onset to minimise stimulus artefact. We recorded the CMAP from active electrodes placed slightly below the midpoint of the muscle (at a location where the cVEMP had the usual polarity, but allowing some space between the stimulating and recording electrodes) and near the sternal insertion point (at a location where the cVEMP was reliably inverted in each subject). The recording sites near the muscle midpoint were 11-14 cm below the mastoid marker (i.e. 1.5-4 cm below the midpoint of the muscle [9.5-10 cm]) and those near the sternal insertion were 16-18.5 cm below the mastoid marker (1.5-4 cm from the bottom of the muscle). The reference electrode was on the sternum. In each case the CMAP was compared to the cVEMP evoked by AC sound using the same electrodes in the same session.

Sonography of SCM muscle

Sonographic examination of the SCM muscle and sternal tendon was performed in a different subset of 5 subjects in a separate session to compare the location of the muscle-tendon junction with the surface response inversion point (3 females, 2 males, 24-54 yrs of age). Testing was performed by a neurologist experienced in ultrasonography (AB) using a high resolution 10-18 MHz linear probe (MyLabOne, Esaote, Italy). Subjects reclined in the supine position and rotated the head away from the SCM of interest to expose the muscle. We examined the same SCM as used above to map the surface responses and focused on the lower half of the muscle. The muscle was viewed in both longitudinal and transverse planes. Under ultrasound guidance the origin, position and length of the lower SCM tendon were measured and compared to the same markers on the skin as used above.

Data analysis

For the surface recordings, we measured cVEMPs at 5 locations: at the midpoint of the SCM, at the last measurement points before the response inverted polarity (for both the upper and lower parts of the muscle) and at the first measurement points at which the cVEMP was inverted. Latencies and amplitudes were measured at the response peaks. Latencies were adjusted to correct for a 0.5 ms delay in the recording system. Amplitudes were measured peak-to-peak (pp, either p13-n23 or n1-p1) and expressed in raw units (μV) or as a ratio of the background contraction (14). The strength of the background SCM contraction was measured over the 20 ms pre-stimulus interval after full-wave rectifying and then averaging the EMG from each frame ('mean rectified EMG').

Our procedure for single motor unit analysis has been described in detail previously (15, 19). Motor unit spikes from single units were identified using a threshold level and clustered with custom software (Matlab, The MathWorks Inc.) based on an automatic algorithm using wavelets and super-paramagnetic clustering (13). In some trials a single unit was recorded, while in others there were multiple units, from which single units were extracted using the sorting algorithm. Peri-stimulus time histograms consisting of 200 bins of 1 ms width and centered at whole numbers were constructed for each unit. The cluster software was set to maximise specificity rather than sensitivity and the accuracy of the software was confirmed for each motor unit by visual comparison of the selected spikes with the raw data. The minimum number of spikes in a histogram was set to 800 to ensure that each histogram had sufficient data to detect a decrease in activity. Based on the spike count measured over the 100 ms pre-stimulus period the 2.5 and 97.5 quantiles were determined and used as threshold levels. Local maxima and minima were accepted if they exceeded these limits and occurred after the first 8 post-stimulus bins. The amplitude of each significant change in firing was expressed as a proportion of the median baseline spike count.

We reported medians and ranges (i.e. minimum and maximum values), except in Figure 2, in which interquartile range is shown. We used non-parametric statistics due to the small number of subjects tested. Comparisons across recording or reference electrode positions were made with Friedman's ANOVA by ranks for related samples, and post-hoc comparisons were performed using the Wilcoxon signed ranks test. For single unit responses, latencies and amplitudes were first averaged for each subject as there were multiple observations made at each recording site. The significance level was $\alpha = 0.05$.

Results

Surface cVEMP recordings

Tracking the cVEMP along the SCM muscle

The cVEMP had the typical positive-negative polarity in each subject when recorded from the midpoint of the SCM muscle. As the recording electrode was moved toward each insertion point, the p13 initially became smaller and the p13 latency increased, then the polarity inverted to a negative-positive waveform (n1-p1). This pattern of responses was seen in all subjects and a representative example is shown in Figure 1.

The SCM muscles were between 16 and 22 cm in length, with a median length of 20 cm. The cVEMP inversion occurred between two *adjacent* recording sites (1 cm apart) in 6/10 and 2/10 subjects for the upper and lower muscles, respectively (e.g. Figure 1, electrodes 1 and 2). In these cases the inversion point was taken as the midpoint of these electrodes. In the remaining cases there was at least one *intermediate* stage at which the reflex was not as well formed and it was not as clear whether the initial peak was a late positivity or an early negativity (Figure 1, electrode 6). In these cases the intermediate stage (or the midpoint of 2 intermediate stages) was taken as the inversion point. The median point of inversion of the reflex at the upper part of the muscle was 20% of the mastoid-sternum distance (range 15-30%, equivalent to a median of 4 cm, range 3-6 cm). At the lower part of the muscle, the

polarity inverted at a median of 76% of this distance (65-85%, 15.1 cm, or 24% from the insertion at the sternum [median 4.1 cm, range 3-7 cm]).

cVEMP peaks were measured at 5 locations: in the middle of the muscle (median 10 cm, range 8-11 cm), at the last recording site before the inversion where the reflex still clearly had the usual polarity (median for upper muscle 5 cm [range 3.5-7 cm], lower muscle 13.6 cm [range 11-17.5 cm]), and at the first location in which the response was clearly inverted (median for upper muscle 3 cm [range 2.5-5 cm], lower muscle 16.1 cm [range 13-18.5 cm]). Amplitude and latency values for the main recording sites are shown in Table 1 and Figure 2. Across these 5 recording sites, there were significant effects of electrode position on nearly all cVEMP components: peak 1 (p13 or n1) latency, peak 2 (n23 or p1) latency, peak 1 amplitude, pp amplitude and mean rectified EMG level ($F_{r(4)}$ = 11.8-26.4, P = 0.000-0.019). The remaining effects, on peak 2 amplitude and the ratio measure of amplitude, showed only trends towards significance (peak 2 amplitude $F_{r(4)}$ = 9.2, P = 0.056; ratio $F_{r(4)}$ = 8.7, P = 0.069).

The p13 latency tended to increase at the last normal recording site, by approximately 3.5 ms at the upper muscle (range 0.4-8.2 ms, Z = -1.9, P = 0.059) and 5 ms at the lower muscle (range 1.6-8.2 ms, Z = 2.2, P = 0.028), but the n1 of the inverted response was not significantly different from the p13 latency at the middle of the muscle. In contrast, the increase in latency for the n23 at the last normal site was not as marked or significant (1.2 ms at the upper muscle, range -0.7-6.6 ms, Z = -1.02, P = 0.308, and 2.8 ms at the lower muscle, range -3-7.2 ms, Z = 1.89, P = 0.059), but the p1 of the inverted response was significantly earlier than the n23 latency at the middle of the muscle (by 3.5 ms at the upper muscle, range -0.6-9.6 ms, Z = 2.7, P = 0.007, and 2.6 ms at the lower muscle (range -1.9-8.3 ms, Z = -2.5, P = 0.013)).

The p13 amplitude at the middle of the muscle was significantly larger than all other sites except the last normal site for the upper muscle (Z = 2.7-2.8, P = 0.005-0.007). The

298 inverted cVEMPs were similar in size to the last normal cVEMP recorded before the point of
299 inversion. Likewise, the pp amplitude was clearly largest at the middle site and nearly twice
300 the size of responses recorded at all other sites (Z = 2.0-2.7, P = 0.007-0.047), which were not
301 different from each other. The rectified EMG was also largest when measured from the
302 middle of the muscle (Z = 2.1-2.8, P = 0.005-0.037 compared to all other sites). A similar
303 pattern was seen for the median values for peak 2 amplitudes and ratios, but as the primary
304 analyses did not reach significance they were not analysed further.
305
306 Reference comparison
307
308 Three of the five active cVEMP recording sites described above were compared across
309 four references sites: the sternum (original reference), the ipsilateral and contralateral earlobes
310 and the contralateral wrist. The active electrode sites were the middle of the muscle and the
311 two locations at which the response was clearly inverted. The results are shown in Table 2
312 and Figure 3. Changing the reference site did not systematically affect the polarity of the
313 reflexes. However, in one subject with small cVEMPs the reflex was abolished at the upper
314 SCM recording site with the contralateral earlobe reference and at the lower site with the
315 contralateral wrist reference. In two further subjects, the reflex at the upper recording site was
316 no longer clearly inverted with the contralateral wrist reference (for one subject) and the
317 ipsilateral earlobe reference (for the other).
318 There were no significant effects of reference site on the latency of the response peaks,
319 except for peak 1 at the lower SCM recording site ($F_{r(3)}$ = 10.9, P = 0.012), where the n1
320 peaks recorded with the contralateral wrist (12.2 ms) and contralateral earlobe references
321 (13.6 ms) were significantly different from each other (Z = -2.8, P = 0.005). In contrast,
322 changing the reference had an effect on most measures of cVEMP amplitude, though the
323 effects reached significance only for the middle and upper recording sites. Only those from
324 the middle recording site are described, as they were the largest. There were significant

effects on all four measures of amplitude (p13 amplitude $F_{r(3)} = 8$, P = 0.045; n23 amplitude $F_{r(3)} = 11.2$, P = 0.011; pp amplitude $F_{r(3)} = 12$, P = 0.007 and ratio $F_{r(3)} = 18.5$, P < 0.001). In all cases the contralateral wrist reference reduced the size of the cVEMP compared to the original sternum reference (Z = 2.0-2.7, P = 0.007-0.041), while there were no significant amplitude differences between the sternum and earlobe references. In addition, the contralateral earlobe reference produced larger responses than both the ipsilateral earlobe reference (Z = 2.1-2.8, P = 0.005-0.037) and the contralateral wrist reference (Z = 2.3-2.8, P = 0.005-0.022). There were no significant differences in background contraction between the reference sites ($F_{r(3)} = 0.36$, P = 0.948).

Single motor unit recordings

We recorded a total of 63 single motor units across the three muscle sites. There was a significant short-latency change in activity in 61 of these units and in each case this was a decrease or gap in firing. We did not find any significant increases of single motor unit activity at any recording site. Table 3 summarises the single motor unit data and representative responses are shown in Figure 4.

In one volunteer there were two units recorded from the middle electrode site which did not respond to the vestibular stimulus, despite the presence of a surface response. Among all subjects, there were 10 single units that showed a brief change in activity before a stronger response, however these false-positive results were equally distributed across both polarities and all electrode sites and were not considered further (decreases in activity: 5 cases, all muscle sites; increases in activity: 5 cases, upper and middle muscle sites).

The decrease in single motor unit activity tended to occur earlier at the middle electrode site (12 ms compared to 15 ms at the upper and lower sites), but this was not significant ($F_{r(2)} = 4.8$, P = 0.091). There were also no significant differences in the size of the decrease in firing between electrode sites ($F_{r(2)} = 3.3$, P = 0.196), however firing was

completely abolished in 61% and 65% of units recorded from the middle and lower recording sites, but only 33% of upper recording sites.

Additional recordings made in one subject at a more distal part of the muscle (3 cm above the clavicle) produced 7 single motor units. In each case there was a significant early gap in firing at a median of 14 ms (13-16 ms).

Comparison of cVEMP and electrically-evoked CMAP

Compound muscle action potentials evoked by electrical stimulation of the muscle were recorded from both the middle and lower electrode sites in all five subjects (Figure 5). The response recorded close to the midpoint of the muscle always had the typical negative-positive polarity, with peaks at 6.2 ms (range 3.4-8.2 ms) and 15 ms (range 11-25.4 ms). Median amplitudes were 965 μV peak-to-peak (range 60-1223 μV). In contrast, the response recorded over the lower part of the muscle near the sternal insertion point had inverted (positive-negative) polarity, with peaks at 7.8 ms (range 6.6-11.4 ms) and 13.2 ms (range 11-20.4 ms) and amplitude 646 μV (range 10-925 μV). In each subject, cVEMPs recorded at the same sites had the expected positive-negative polarity near the midpoint and inverted polarity over the lower part of the muscle, as described above. cVEMP amplitudes and latencies near the middle of the muscle were 99 μV (range 82-196 μV), 14.8 ms (range 13.8-18.4 ms) and 22.4 ms (range 21.4-28.4 ms), slightly later than those reported above, as the electrodes were positioned several cm below the midpoint to minimize stimulus artefact. Corresponding values near the sternal insertion were 101 μV (range 54-151 μV), 13.0 ms (range 11.8-14 ms) and 20.8 ms (range 17.8 ms).

SCM tendon measurement

Sonographic measurement in 5 volunteers revealed that the sternal tendon of SCM began just below the midpoint of the muscle, at approximately 60% of the total muscle-tendon length (range 47-68%), measured as described above. The tendon began deep within the muscle and gradually became superficial as it was tracked downwards toward the insertion point (Figure 6). The point at which the tendon reached the muscle surface was 71% of the total muscle-tendon length (range 63-74%). Muscle was typically seen below the tendon as far down towards the insertion point behind the medial clavicle as could be viewed. A measure of the total tendon length was not possible using this method as the portion of tendon beneath the clavicle could not be viewed in the same plane as that above the clavicle. The median cVEMP inversion point in these subjects was 74% of the total length (range 65-85%). This placed the inversion point below the tendon origin in all subjects and below the point at which the tendon became superficial in 3/5 subjects. In the remaining 2 subjects, the inversion point was at or (4%) above the point where the tendon reached the surface (probably within the measurement error of the technique).

Discussion

We investigated the polarity of the cVEMP in normal subjects and found that in each subject there were systematic changes in the reflex as the recording electrode was moved away from the SCM muscle belly and toward the insertion points. With growing distance from the motor point, cVEMP latencies became increasingly prolonged and eventually the reflex inverted and began with a negativity instead of the usual p13 positivity. This occurred at both the upper part of the muscle near the mastoid process and at the lower anterior part of the muscle near the sternal insertion point. In contrast, when we recorded the activity of underlying single motor units from the upper, middle and lower parts of the muscle, we found a decrease, or inhibition, of firing at each recording site in all 5 subjects. We did not record

any significant increases in firing. The results suggest that the reflex has constant polarity along the entire sternomastoid muscle and do not support Wei et al.'s (20) hypothesis of contrasting vestibular projections to different parts of the muscle.

Using surface recordings, we reproduced Wei et al.'s (20) finding that the cVEMP is inverted when recorded over the lower part of the SCM. However, we additionally found a similar polarity inversion at the upper part of the muscle, while Wei et al. (20) recorded responses with the usual polarity. This might have been due to small differences in measuring the SCM muscle length between the studies and the fact that Wei et al. (20) recorded at only three fixed locations. In the current study we tracked the potentials along the whole muscle and likely recorded closer to the insertion points at both ends. Previous data has suggested that the motor point is closer to the mastoid SCM insertion point, i.e. at 56-69% of the sternoclavicular-mastoid process distance (3). This could mean that the upper electrodes in Wei et al. (20) were closer to the motor point than those over the lower muscle quarter. Apart from the differences in reflex polarity, the data reported by Wei et al. (20) are consistent with those reported here. The inverted VEMPs at the lower recording sites in both studies had peak latencies similar to or earlier than those in the muscle middle. The latency of the non-inverted cVEMP at the upper site in Wei et al. (20) was later than that in the muscle middle, suggesting that this recording site was just below the reflex inversion point.

In the current study, we showed that the reflex inversion in the SCM muscle also applied to the compound muscle action potential evoked by direct electrical stimulation of the muscle and is therefore not specific to the cVEMP or vestibular-dependent reflexes. Our data additionally showed that the polarity inversion of the cVEMP was unrelated to the position of the *reference* electrode. Using the sternum reference, the pattern of amplitude, latency and polarity changes was the same for both the upper and lower parts of the muscle, despite the fact that the upper recording electrode was always further from the reference. Similar to Wei et al. (20), who compared a sternum and wrist reference, we used 3 reference sites in addition

427 to the original sternal reference and found that, although there were systematic differences in
428 the size of the cVEMP, the polarity of the reflex remained constant. The polarity inversion is
429 instead likely to be caused by changing the position of the active *recording* electrode.
430 Previous studies have shown that polarity inversions of the compound muscle action
431 potential can occur when the recording electrodes are placed close to the muscle tendons (e.g.
432 9). Lateva et al. (9) recorded CMAPs from the thenar muscle of the hand and showed that the
433 CMAP began with a positivity instead of the usual negativity when recorded with an electrode
434 over the tendon and referred to one just beyond the tendon. Standing waves are known to be
435 generated when a travelling source reaches a boundary or sudden change in resistance in the
436 surrounding tissue, such as a tendon (7, 8). However, it is unclear to what extent the
437 proximity of the recording electrode to the muscle-tendon junction *per se* played a role in this
438 inversion effect. First, our measurements of the sternal SCM tendon suggested that the
439 muscle-tendon junction is dispersed over a wide area of muscle. The tendon appears to begin
440 near the longitudinal midpoint of the muscle, toward the centre of the muscle belly, and
441 gradually makes its way to the muscle surface, becoming thicker toward the insertion point.
442 The overall tendon length was consistent with surgical reports, in which tendons of at least 8
443 cm have been described (1, 11). The muscle-tendon junction was therefore likely to be spread
444 across a large area instead of a limited region. This might mean that the surface potential
445 inverted only once the recording electrode was positioned beyond the point where most
446 muscle fibres had terminated, however, muscle fibres could be viewed along the entire length
447 of the SCM, including below the clavicle. Second, if the inverted response was generated by
448 a travelling wave reaching the muscle-tendon junction, it would be expected to occur later
449 than the wave generated at the motor point (17). Instead, while the p13 became
450 systematically delayed with increasing distance from the motor point, the latency of the
451 inverted cVEMP was similar to that of the normal reflex recorded near the motor point. The
452 changes might therefore be explained by simple volume conduction effects, as suggested by

Wei et al. (20), whereby the recording electrode is located sufficiently far from an active source that it records the standing wave at the time it is generated (17). It is possible that the tendon plays a role in this, as the point of reflex inversion was invariably below the start of the tendon, and was near the point at which the tendon became superficial. The tendon is likely to be a high impedance tissue and, unlike muscle, is inexcitable. The gradual loss of overlying muscle might potentially allow distant standing waves to dominate the recording (17). The close proximity of recording and reference electrodes near the sternoclavicular junction would have been expected to reduce the impact of far-field effects on the recording, although it may be that the difference in impedance of the two recording sites was sufficient to reveal the standing wave.

Our data are consistent with previous reports providing evidence that the cVEMP originates from a single motor point and spreads gradually along the muscle (3, 10). Colebatch (3) found that the p13 became later with increasing distance from the motor point, while changes to the n23 potential were not as marked, and our data support this. The current single unit data was also consistent with this latency effect, but did not reach significance due to large variability in the timing of the inhibition. Overall, the data suggest that the p13 behaves as a travelling wave, produced by the inhibition as it begins at the motor point and moves along the muscle in both directions. The n23 potential is more like a combination of phenomena, including a trailing dipole created following the propagating inhibition, a standing wave generated by the inhibition reaching the end of the muscle and a small rebound in firing following the inhibition (15). The potential recorded at any point along the muscle will be a combination of these waveforms (8). It is also possible that cVEMP electrodes pick up activity from nearby muscles. In particular, when recording electrodes are placed too close to the mastoid insertion point of the SCM, activity from the post-auricular muscle (PAM) reflex could contribute to the inverted reflex, given its excitatory polarity (12).

Clinical considerations

Our results show that the surface response can be ambiguous and may not always reliably indicate the polarity of the reflex in the underlying muscle, especially if the recording electrode is placed away from the motor point. There is variability in the location of the motor point across subjects (3, 10), and we also found considerable differences in the point of reflex inversion. In two subjects, the inversion point was only 3 cm away from the measured midpoint of the muscle. Careful placement of the recording electrode near the middle of the muscle (or just above this point, near the motor point) is required to ensure the most accurate measurement of reflex polarity, amplitude and latency. However, as the locations of the motor and inversion points in individual patients will not be known in clinical contexts, small errors in electrode placement with respect to the motor point are likely to be inevitable. Of particular concern is the change in latency that occurs before the reflex inverts, as an apparent latency delay may be erroneously interpreted as significant without raising suspicion of an electrode placement effect. Caution is therefore warranted in interpreting latency delays.

Our results suggest that the typical medial clavicle or sternal reference electrode may not be completely indifferent, as recording electrodes placed near the sternoclavicular joint produced clear reflexes. However, a reference over bone is preferable to one directly over muscle or tendon, as bone has higher impedance and is likely to be a better indifferent. Regardless, as the reflex polarity at these locations is opposite to that recorded over the muscle midpoint, use of the traditional montage will serve to enhance the cVEMP amplitude. In contrast, we showed that an indifferent reference at the wrist produced smaller reflexes, which would be less suitable for clinical use. We therefore recommend continued use of the traditional belly-sternum/clavicle cVEMP montage.

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Figure Captions

Figure 1. Effect of surface recording electrode position on the cVEMP in a single subject. Recordings from 7 different points along the muscle are shown: the midpoint (site 4, black trace), near the insertion point of the upper part of SCM with the mastoid (sites 1-3) and near the insertion of the anterior arm with the sternum (sites 5-7). The reference was placed on the upper sternum. Electrode positions are given in % of total mastoid-sternum distance, which was 19 cm in this subject. At the midpoint (site 4), a typical positive-negative waveform with p13-n23 latencies was seen. With increasing distance from the midpoint the cVEMP became smaller and latencies prolonged (dark grey traces, sites 2, 3, 6 and 7). As the electrodes approached the insertion point of the muscle the response inevitably inverted and had a negative-positive polarity (light grey traces, sites 1 and 7). The initial peak latency of the inverted response was similar to the p13 latency.

Figure 2. Summary of the changes in polarity, latency and amplitude of cVEMPs measured at different points along the SCM muscle. Medians and interquartile ranges are shown for electrodes at the midpoint, at the last position with positive-negative polarity and near the insertion of the anterior SCM with the mastoid and sternum, where responses had inverted, negative-positive polarity. Surface positivities (i.e. p13 responses) are indicated by an encircled plus (+) sign, while negativities (i.e. n1) are indicated by a minus (-) sign. The responses changed size, latency and polarity as the electrode was moved along the muscle. The largest and earliest responses were recorded at the middle of the muscle and always had positive-negative polarity. As the recording electrode was moved toward the mastoid and sternal insertion points, cVEMPs appeared later and became smaller The polarity of the cVEMP then reversed. Amplitudes of the inverted responses (-) were similar to the last recorded responses with regular polarity, while latencies matched those of the middle of the muscle.

Figure 3. Effect of reference electrode placement on the cVEMP in a single subject. Reference position had no systematic effect on the polarity or latency of the reflex, but modulated the amplitude of the response. Four different references were used to compare cVEMPs recorded from the upper, middle and lower parts of the muscle. Reference positions were sternum (standard position), contralateral wrist, contralateral earlobe (C. Ear) and ipsilateral earlobe (I. Ear). Responses in the middle had the usual positive-negative polarity, while those at the upper and lower positions near the insertion points had inverted negative-positive polarity.

Figure 4. Effect of needle electrode location in a single subject. Single motor units were recorded with concentric needle electrodes from the middle of the SCM muscle and at the upper and lower parts of the anterior component of the muscle, as close to the tendon as possible. There was a significant decrease or gap in single motor unit activity at all sites following the stimulus: at a median of 12 ms in the middle of the SCM muscle and 15 ms near each of the tendons. This suggests that the reflex is inhibitory in nature along the whole SCM muscle. Surface responses were recorded from the same subject in a separate session from electrodes placed over similar locations and referred to the sternum. The surface response had the typical positive-negative (p13-n23) polarity when recorded near the middle of the muscle, but was inverted (with negative-positive polarity, but similar peak latency) when recorded at the upper and lower parts of the muscle. Surface responses are unreliable indicators of reflex polarity when recorded away from the muscle midpoint.

Figure 5. Comparison of (A) electrically-evoked compound muscle action potentials (CMAPs) and (B) sound-evoked cVEMPs in a single subject. This comparison demonstrated that the polarity inversion was not specific to vestibular reflexes in SCM muscle. Part A shows the CMAP (black traces) recorded at two locations during stimulation over the motor point: 14 cm below the mastoid (i.e. 4 cm below the midpoint of the muscle and a short distance from the stimulating electrode to minimize stimulus artefact) and 18.5 cm: below the point of cVEMP reflex inversion. Grey traces show the result of using less intense current below threshold, which produced no response and only stimulus artefact (beginning just before 0 ms when the artefact eliminator was switched on). When recorded close to the muscle belly the CMAP had the expected negative-positive polarity, while close to the sternal insertion point the response was inverted and began with a positive peak. Part B shows cVEMPs recorded in the same session using the air-conducted sound stimulus. The response near the middle of the muscle had the typical positive-negative polarity and that recorded near the insertion of the muscle at the sternum showed the polarity reversal illustrated in prior figures. Note the different amplitude scales in parts A and B.

Figure 6. Ultrasonography of the right SCM muscle. Ultrasound measurements were made to compare the position of the SCM muscle-tendon junction with the site of surface reflex inversion. Part A shows a transverse view of the right SCM near the top of the sternal tendon. The dotted lines show the area of SCM, while the arrows point to the position of the tendon. The tendon began toward the centre of the muscle and became superficial (shown in Part B) as it was followed down toward the sternal insertion point. Part C shows an idealised lateral view of tendon position within the muscle, along with data from the 5 subjects regarding the measured landmarks. The cVEMP inversion point was below (in all subjects) the tendon origin and close to (2/5 subjects) or below (3/5 subjects) the point at which the tendon became superficial. The letters A and B shown in Part C indicate the approximate levels at which the ultrasound images in Parts A and B were taken.

678 **Table1.** Effect of changing recording electrode position on cVEMP properties.
679

Electrode position	Inverted upper	Last regular upper	Middle	Last regular lower	Inverted lower
Polarity	n-p	p-n	p-n	p-n	n-p
p13/n1 Lat*	12.0 (9.8-13.6)	16.2 (12.7-21.5)	12.7 (11.7-13.4)††	17.7 (13.8-20.0)	12.5 (11.3-13.0)
p13/n1 Amp†	22.0 (10.0-46.2)	30.6 (18.8-109.8)	45.8 (12.1-107.9)	30.8 (14.5-63.0)	21.3 (0-69.9)†††
n23/p1 Lat	18.3 (17.1-20.8)	23.0 (18.6-29.4)	21.8 (17.4-28.0)	24.6 (22.9-27.5)	19.2 (16.1-20.4)
n23/p1 Amp	36.1 (7.0-79.9)	28.9 (7.6-107.8)	56.8 (22.3-99.0)	27.5 (2.8-117.2)	30 (17.7-109.6)
Amp (pp)	57.5 (17.0-126.1)	61.8 (23.9-217.6)	111.9 (43.0-167.3)	51.6 (19.4-180.03)	51 (20.6-179-5)
Amp (ratio)	0.88 (0.47-1.86)	0.92 (0.29-1.55)	1.16 (0.34-1.96)	0.79 (0.24-1.44)	0.66 (0.32-1.64)
Contraction	65.7 (39.7-102.9)	73.3 (42.5-140.5)	91.6 (59.0-161.2)	73.6 (47.0-134.4)	74.6 (42.8-109.3)

680
681 All values are median (range). * - Latency is given in ms, † - Amplitude is given in μV using absolute values. †† - plus an outlier of 18.6 ms. ††† -
682 the 0 results was measured from a baseline-shifted response, not an absent response.
683
684
685

686 **Table 2.** Effect of changing reference electrode position on cVEMP properties.
687

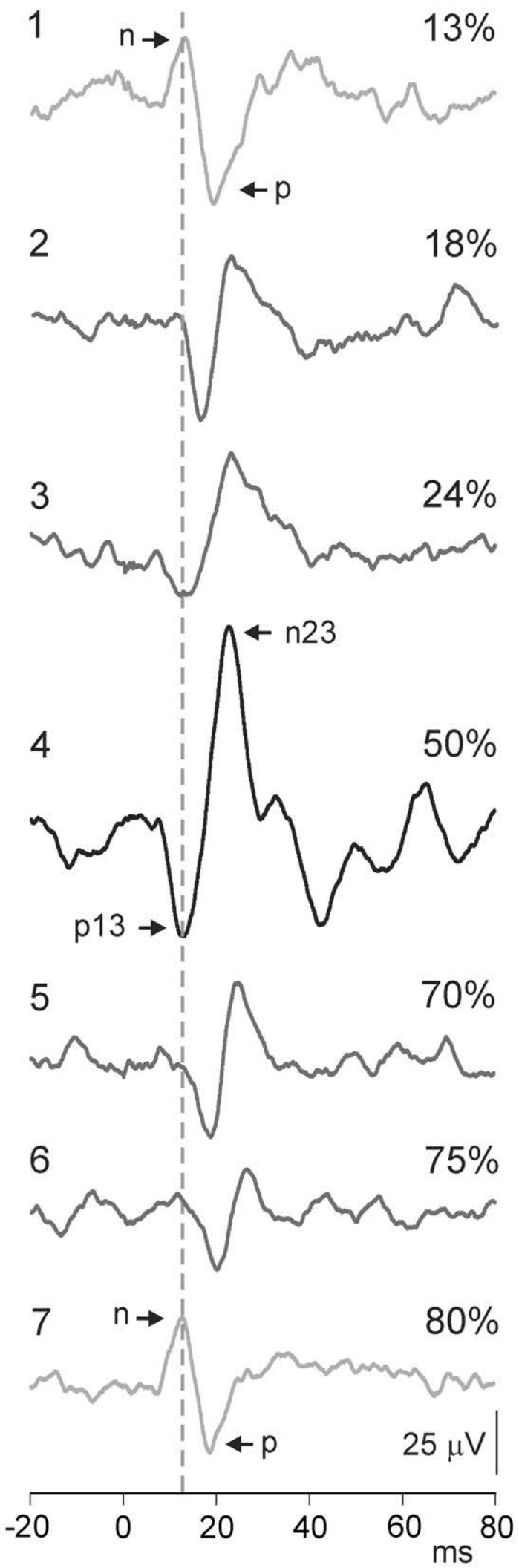
Inverted upper electrode (n1-p1)				
Electrode position	Sternum	Contra wrist	Contra earlobe	Ipsi earlobe
p13/n1 Lat*	12.0 (9.8-13.6)	12.6 (11.0-13.7)	12.9 (11.8-14.6)	12.8 (11.7-14.2)
p13/n1 Amp†	22.0 (10.0-46.2)	24.3 (8.4-40.2)	26.9 (11.6-68.7)	14.0 (4.5-50.4)
n23/p1 Lat	18.3 (17.1-20.8)	19.4 (17.1-22.7)	18.9 (17.5-22.2)	18.8 (17.6-21.4)
n23/p1 Amp	36.1 (7.0-79.9)	21.4 (13.9-71.4)	50.4 (13.7-84.1)	24.0 (13.7-77.1)
Amp (pp)	57.5 (17.0-126.1)	46.2 (28.0-46.2)	70.0 (25.3-141.2)	38.6 (21.3-127.5)
Amp (ratio)	0.88 (0.47-1.86)	0.74 (0.55-1.25)	1.28 (0.63-2.08)	0.77 (0.41-2.07)
Contraction	65.7 (39.7-102.9)	67.9 (39.3-137.9)	62.2 (40.2-105.2)	54.3 (23.9-108.6)
Middle electrode (p13-n23)				
Electrode position	Sternum	Contra wrist	Contra earlobe	Ipsi earlobe
p13/n1 Lat*	12.7 (11.7-13.4)††	13.0 (11.5-17.9)	12.5 (10.6-17.7)	12.5 (11.9-18.9)
p13/n1 Amp†	45.8 (12.1-107.9)	28.9 (12.3-60.2)	61.3 (15.9-168.1)	35.2 (5.4-93.9)
n23/p1 Lat	21.8 (17.4-28.0)	22.3 (17.5-29.2)	22.3 (17.9-29.3)	23.1 (17.2-26.2)
n23/p1 Amp	56.8 (22.3-99.0)	47.8 (9.3-79.9)	66.3 (30.8-94.6)	37.1 (12.6-99.6)
Amp (pp)	111.9 (43.0-167.3)	76.5 (27.7-140.1)	124.4 (46.7-262.7)	84.7 (21.3-193.6)
Amp (ratio)	1.16 (0.34-1.96)	0.77 (0.24-1.34)	1.30 (0.44-2.1)	0.77 (0.18-1.85)
Contraction	91.6 (59.0-161.2)	88.5 (65.9-169.9)	85.2 (48.1-218.7)	85.5 (29.6-228.6)
Inverted lower electrode (n1-p1)				
Electrode position	Sternum	Contra wrist	Contra earlobe	Ipsi earlobe
p13/n1 Lat*	12.5 (11.3-13.0)	12.2 (11.3-13.3)	13.6 (10.2-15.2)	12.6 (9.7-14.1)
p13/n1 Amp†	21.3 (0-69.9)	25.2 (9.35-40.4)	24.7 (10.2-55.0)	16.6 (3.7-42.0)
n23/p1 Lat	19.2 (16.1-20.4)	20.0 (17.8-29.0)	20.1 (14.1-22.7)	19.5 (16.7-21.2)
n23/p1 Amp	30.0 (17.7-109.6)	25.2 (12.8-49.0)	26.9 (7.5-109.3)	29.8 (14.6-51.4)
Amp (pp)	51 (20.6-179-5)	50.1 (24.5-89.4)	51.1 (24.9-163.9)	45.0 (20.5-93.4)
Amp (ratio)	0.66 (0.32-1.64)	0.63 (0.43-1.16)	0.85 (0.37-1.51)	0.63 (0.28-1.37)
Contraction	74.6 (42.8-109.3)	76.8 (36.8-106.2)	70.9 (36.7-162.9)	69.4 (36.6-119.7)

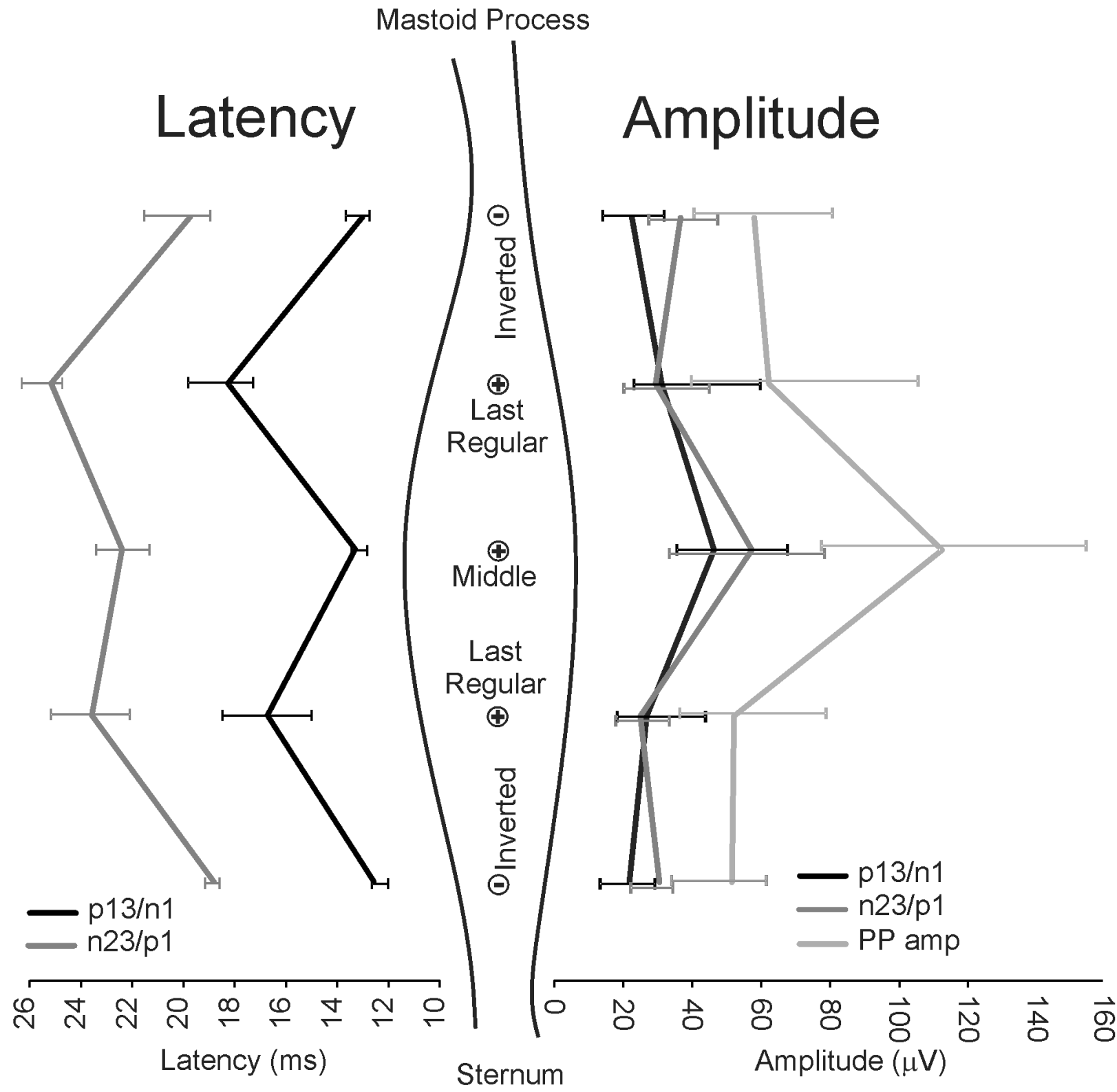
688
689 All values are median (range). * - Latency is given in ms, † - Amplitude is given in µV using
690 absolute values. †† - plus an outlier of 18.6 ms.
691
692
693
694

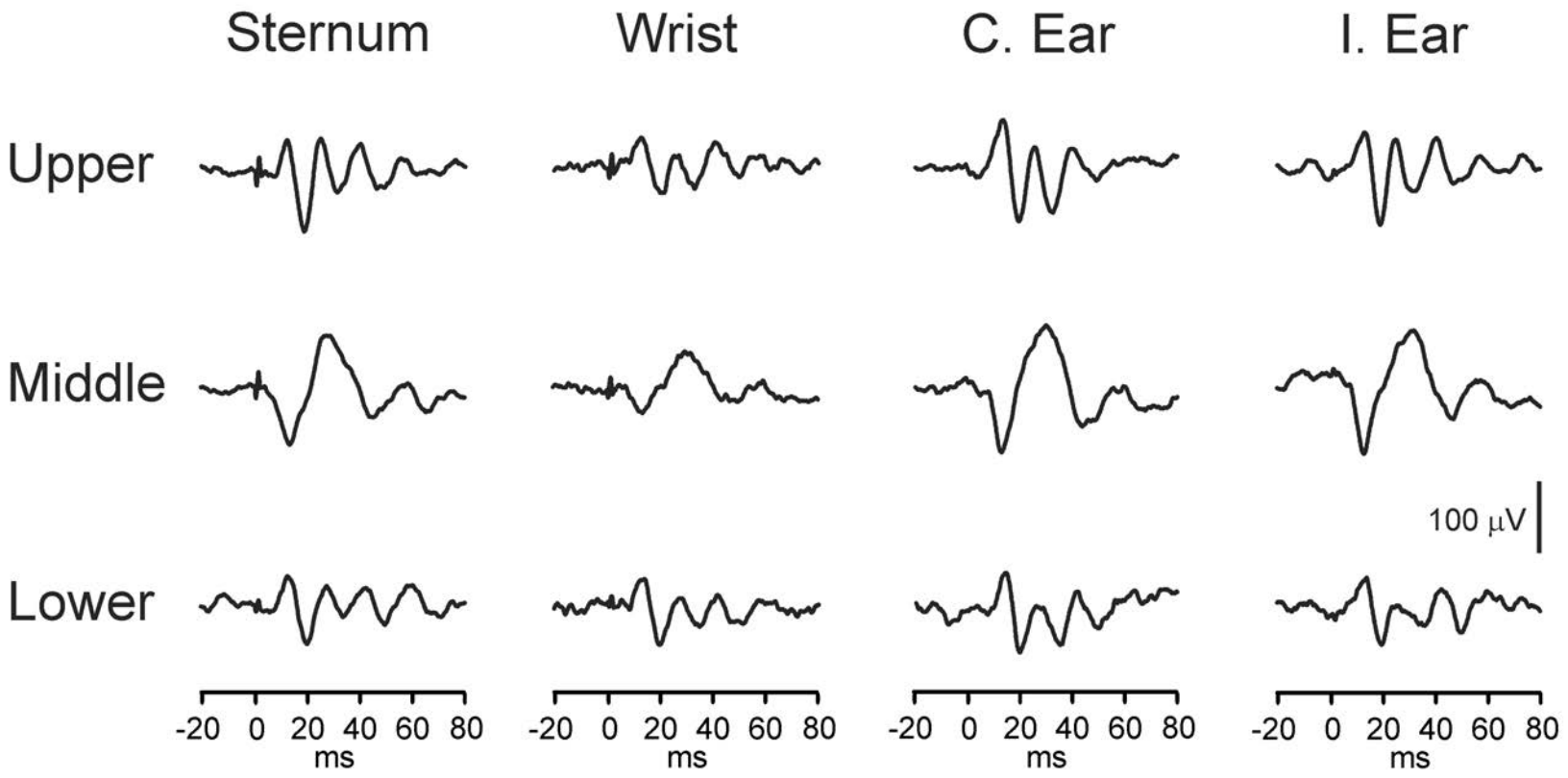
695 **Table 3.** Properties of single motor unit responses recorded at three sites along the SCM
696 muscle.
697

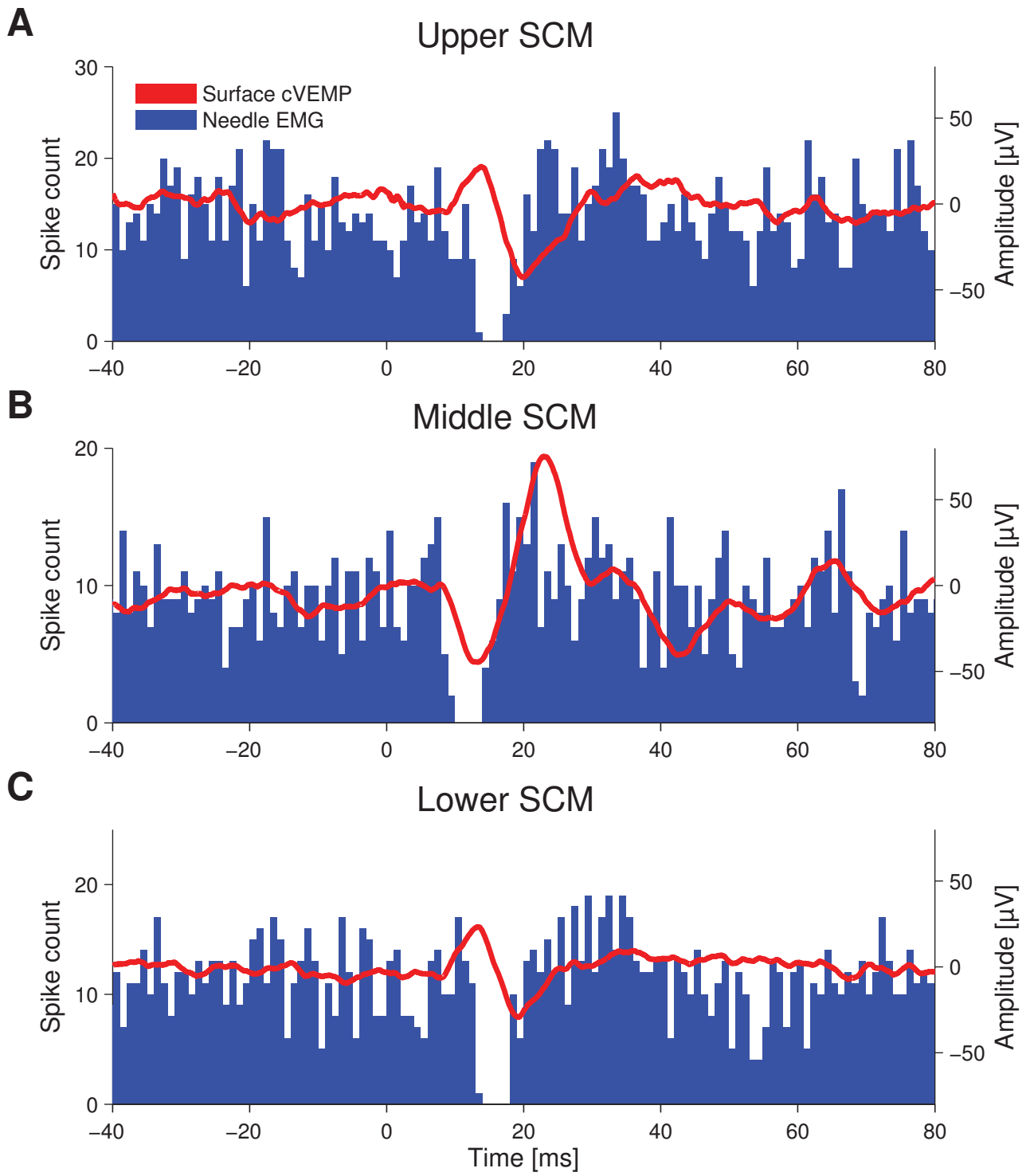
	Upper SCM	Middle SCM	Lower SCM
Latency Median*	15 (9-27)	12 (9-21)	15 (10-25)
Amplitude Median†	0.13 (0.00-0.33)	0.00 (0.00-0.45)	0.00 (0.00-0.36)
N Response	21	23	17

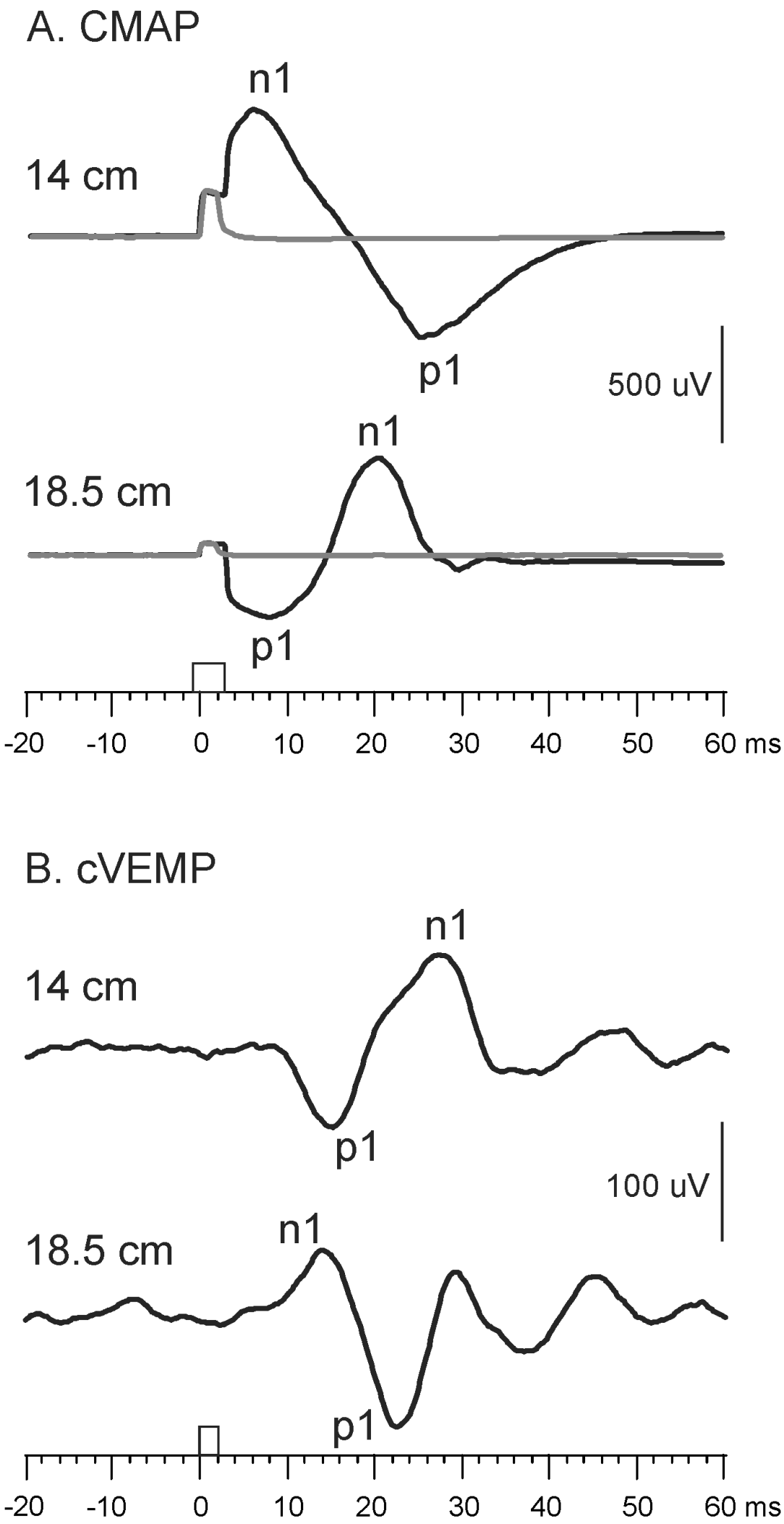
698
699
700 All values are median (range). * - Latency was measured in 1 ms histogram bins. † -
701 Amplitude is given as a proportion of the median baseline spike count.


1
n
13%
p
2
18%
3
24%
n23
4
50%
p13
5
70%
6
75%
7
n
80%
p
25 μV
-20
0
20
40
60
80
ms

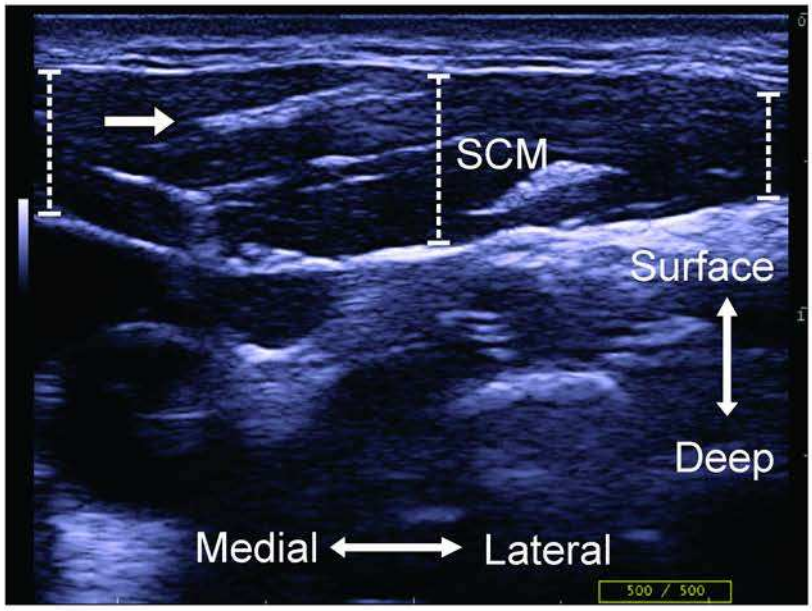

Mastoid Process
Latency
Amplitude
Inverted
Last Regular
Middle
Last Regular
Inverted
p13/n1
n23/p1
PP amp
26
24
22
20
18
16
14
12
10
0
20
40
60
80
100
120
140
160
Latency (ms)
Amplitude (μV)
Sternum


Sternum
Wrist
C. Ear
I. Ear
Upper
Middle
Lower
100 μV
-20 0 20 40 60 80
ms

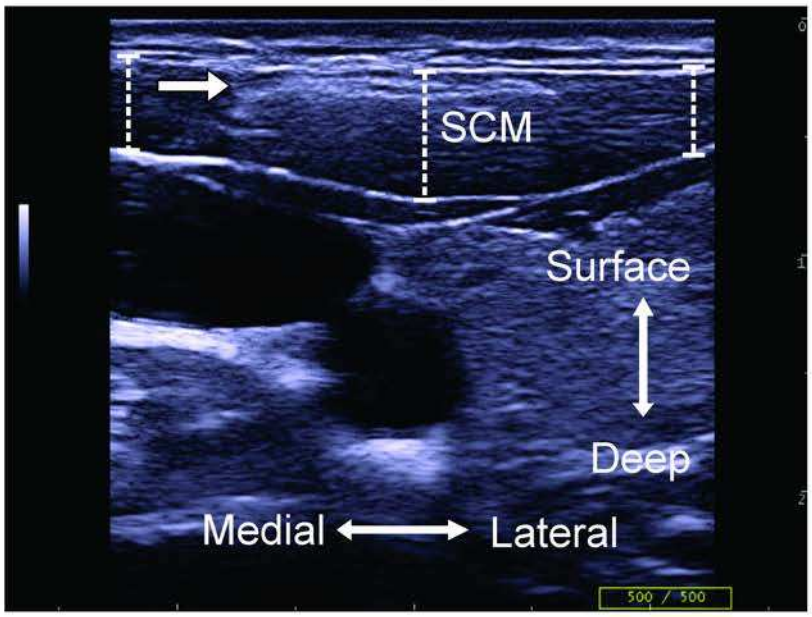

A
Upper SCM
Surface cVEMP
Needle EMG
Spike count
Amplitude [µV]
B
Middle SCM
C
Lower SCM
Time [ms]


A. CMAP
n1
14 cm
p1
500 uV
n1
18.5 cm
p1
-20
-10
0
10
20
30
40
50
60 ms
B. cVEMP
n1
14 cm
p1
100 uV
n1
18.5 cm
p1
-20
-10
0
10
20
30
40
50
60 ms

A. Tendon origin



B. Tendon becomes superficial



C. Diagram of tendon position